

## Nitrogen Cycle

Nitrogen fixation :

This process in which nitrogen ( $N_2$ ) in the atmosphere is converted into ammonia ( $NH_3$ ) fixed form, by a number of soil microorganisms are capable of producing an enzyme called nitrogenase which needs the metal molybdenum and iron to complete the process. This process is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides for DNA and RNA and amino acids for proteins.

Two types of bacteria are responsible for fixing nitrogen :

Family 1 : Azotobacteriaceae      ex. *Azotobacter*.

Family 2 : Rhizobiaceae      ex. *Rhizobium*.

Some types of bacteria can fix nitrogen but are less efficient such as *Clostridium*, *Klebsiella*, Cyanobacteria.

Nitrogen fixation can possibly be done in two ways:

1- Symbiotic Nitrogen fixation . ex. *Rhizobium*.

2- Non-Symbiotic Nitrogen fixation (free living). ex. *Azotobacter* sp., *Azospirillum*

**Symbiotic Nitrogen fixation :--- ex. *Rhizobium***

Nitrogen fixation through nodule formation in Leguminous plants

Leguminous plants bearing nodules on their roots as a result of injury to root hairs with bacteria *Rhizobium*, in large numbers inside these root nodules. Bacteria are parasites on the plant where they get the necessary energy for their growth and fixed nitrogen that is useful for the plant. The symbiotic bacteria Rhizobia (from the Greek words Riza = Root and Bios = Life)

Procedure:

- 1- Take a plant called sweet clover (*Melilotus*) from soil.
- 2- Cut roots & wash them well with tap water to remove soil particles.

- 3- Cut large pink or red nodules true nodules (near the root) , False nodules Located far from root.
- 4- Put true nodules on clean slide and smash it with another slide , leave it to dry and stain with gram pigment .
- 5- Examine under oil immersion , *Rhizobium* appear look like latin letters Y , X, Z, T .

#### **Non-Symbiotic Nitrogen fixation (free living):**

The fixation of free nitrogen in the soil can gets by most microorganisms living freely or outside the plant cell that called non-symbiotic N<sub>2</sub> fixation. It is performed by the aerobic and anaerobic bacteria and blue green algae.

The process involved microorganism in soil free-living *Azotobacter chroococcum* bacteria , this bacteria G-ve, motile , often pleomorphic ranging from rods to cocci shapes , strict aerobic, the colonies appear like sticky mucus because they around by cyst , when bacteria grown on agar media form large convex , mucoid colonies , with brownish color, growth in PH (7.2-8) .To isolation this bacteria use (Nitrogen –free glucose medium) ,this contains sources carbon, molybdenum .

This bacteria produce two type of pigments :Use to distinguish between 6 types of *Azotobacter*

1-Water- soluble pigments. ( It's the important because it spread in culture medium).

2-Water-in soluble pigments.

Procedure:

1-Transfer 1gm of soil to Nitrogen –free glucose broth medium that packaged in glass bottles.

2- Close the bottles not tight to provide enough aeration and get N<sub>2</sub> from air .

3- Incubate at 30c for 7days in horizontal position to increase the surface area of culture medium that exposed to the air .

4- after incubation :transfer 0.1 ml from Nitrogen –free glucose broth to Nitrogen –free glucose agar and streaking to show colony shape and color water- soluble pigment.